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(21) International Application Number: PCT/US97/16366 (22) International Filing Date: 15 September 1997 (15.09.97) (30) Priority Data: 08/725,194 13 September 1996 (13.09.96) US (71) Applicant: NUTRAMAX LABORATORIES, INC. [US/US]; 5024 Campbell Boulevard, Baltimore, MD 21236 (US). (71)(72) Applicants and Inventors: HENDERSON, Robert, W. [US/US]; 2807 Shady Grove Court, Baldwin, MD 21013 (US). HAMMAD, Tarek [EG/US]; 10 Korada Court #2B, Baltimore, MD 21244 (US). (74) Agent: CALIA, Kurt, Geoffrey; Covington & Burling, 1201 Pennsylvania Avenue, N.W., P.O. Box 7566, Washington, DC 20044 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: SELENIUM AND S-ADENOSYLMETHIONINE NUTRITIVE COMPOSITION (57) Abstract Novel nutritional supplement compositions containing selenium in combination with S-adenosylmethionine (SAM), and, optionally, vitamin B ₁₂ and cyanide, methods of administering these compositions, as well as methods of increasing the methylation of selenium are disclosed. These nutritional supplements have application both in the human and veterinary fields.		

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SELENIUM AND S-ADENOSYLMETHIONINE NUTRITIVE COMPOSITION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to nutritional supplements to the mammalian diet, and more specifically to nutritive compositions that include a combination of selenium and S-adenosylmethionine (SAM), and which optionally include vitamin B₁₂ and cyanide.

2. Background of the Invention

Historically, selenium has been regarded as toxic to animals and humans. Toxic organic forms of selenium include selenium accumulator plants that incorporate selenium into nonproteinaceous amino acids including Se-methylselenocysteine, selenocystathione, selenocystine, and selenohomocysteine. Nonaccumulator plants, on the other hand, incorporate selenium into the proteinaceous amino acid selenomethionine. In addition, both types of plants, as well as certain microflora, detoxify some selenium through methylation, producing dimethylselenide or dimethyldiselenide. It is understood that inorganic forms of selenium are less toxic than organic forms. In presently known mammalian selenium proteins and enzymes, selenium is generally in the form selenocysteine.

In the late 1950s, the medical community discovered that selenium, which had been regarded as a poison and is among the most toxic of all minerals, was an essential biological trace

element in animals and humans. In 1957, Klaus Schwarz, at the National Institutes of Health, discovered that selenium was required by rats to prevent dietary liver necrosis. Later, selenium was found to be a component of glutathione peroxidase, a common mammalian enzyme that functions to protect cells against oxidative damage. Selenium is a necessary part of the diet because of its role in essential enzymes such as glutathione peroxidase. Indeed, a lack of selenium is now understood to have deleterious effects on mammalian health. Low levels of selenium has been associated with an increased risk of certain types of cancers, congestive cardiomyopathy, skeletal myopathy, anemia, cardiovascular disease, immune system dysfunction, and nail and hair abnormalities. [Bonomini and Albertazzi, Artificial Organs, 19(5):443-48 (1995)]. It is understood that, in mammals, methylated forms of selenium are more active than non-methylated forms.

Daily selenium intake in amounts greater than the levels ingested in the typical diet and less than levels required to produce a toxic effect is known to have numerous health benefits including possible reduction in risk of certain types of cancer, a reduction in the effects of various mutagenic agents, a reduction in the toxicity of heavy metals such as cadmium and mercury, the stimulation of the immune system, the detoxification of certain enzyme reactions, and the prevention of cardiomyopathy.

The dietary requirement for selenium may be fulfilled from the inorganic salts, selenate and selenite, and various organic selenium compounds, which are metabolized in part into

selenoproteins.

Animals can metabolize both inorganic and organic forms of selenium and convert monomethylated selenium to its methylated forms. Selenium occurs in a variety of different chemical forms in plants and animals. Plants take up inorganic selenium and convert it to numerous selenium analogs of sulfur compounds whereas animals metabolize both organic and inorganic forms into biologically active compounds. Since animals typically have limited reserves of selenium, it is an essential component of the diet if a sufficient amount of biologically active selenium is to confer its known health benefits.

It has been reported that approximately 250-300 μg per day of selenium is believed to be needed for selenium to play a chemopreventive role. [Hocman, Int. J. Biochem., 20(2):123-32 (1988)]. It has been reported that early symptoms of toxicity occur at about 900 μg per day of selenite, and that about 600-800 μg per day is considered safe. These reports of selenium safety and toxicity relate to an inorganic form of selenium administered without any mollifying agents. As disclosed herein, such a mollifying agent may be S-Adenosylmethionine, vitamin B₁₂ or cyanide.

For humans, an uptake of 50-200 $\mu\text{g}/\text{day}$ is considered safe and adequate by the Food and Nutrition Board, with healthy North American males requiring 80 $\mu\text{g}/\text{day}$ and females requiring 57 $\mu\text{g}/\text{day}$. Actual daily uptakes vary widely around the world dependent upon the concentrations of selenium in soil which varies considerably at different geographical locations.

S-Adenosylmethionine (SAM) is a significant physiologic

compound which is present throughout body tissue and takes part in a number of biologic reactions as a methyl group donor or as an enzymatic activator during the synthesis and metabolism of hormones, neurotransmitters, nucleic acids, phospholipids, and proteins. SAM may be second only to adenosine triphosphate (ATP) in the variety of reactions in which it is a cofactor. SAM is metabolized via three metabolic pathways of transmethylation, transsulfuration, and aminopropylation. [Stramentinoli, Am. J. Med., 83(5A):35-42 (1987)]. In higher organisms, SAM plays a significant role in transmethylation processes with more than 40 anabolic or catabolic reactions involving the transfer of the methyl group of SAM to substrates such as nucleic acids, proteins, and lipids, among others. Also, the release of the methyl group from SAM is the start of a "transsulfuration" pathway that produces all endogenous sulfur compounds. After donating its methyl group, SAM is converted into S-adenosylhomocysteine, which in turn is hydrolyzed to adenosine and homocysteine. The amino acid cysteine may then be produced from the homocysteine. The cysteine thus produced may exert a reducing effect by itself or as an active part of glutathione, which is a main cell anti-oxidant. [Stramentinoli, cited above].

Vitamin B₁₂ is known to function as a coenzyme in biochemical reactions such as the synthesis of proprionic acid and of methionine.

3. Description of Background Art

While all the mechanisms by which selenium provides benefits to the human body are not known, selenium is believed to function as an antioxidant, immune response stimulator, and as a key part of some enzyme detoxification reactions. It is also understood that methylated metabolites of selenium may play an active role in cancer prevention. [Ip and Ganther, Cancer Res., 50:1206-11 (1990)]. It is further understood that the degree of methylation is an important factor affecting the anticarcinogenic activity of selenium, the monomethylated form being the most effective. [Ip et al., Cancer Res., 51:595-600 (1991)]. Accordingly, attempts to supplement the human diet with selenium are known in the art. [See, e.g., Kiremidjian-Schumacher and Stotzky, Environmental Res., 42:277-303 (1987); Shamberger, Mutation Res., 154:29-48 (1985)]. As another example, conventional selenium compounds such as sodium selenite, sodium selenate, and to a lesser extent selenocystine and selenomethionine have been used successfully as dietary supplements for suppression of tumors in rodents. [Vadhanavikit et al., Xenobiotica, 23(7):731-745 (1993)].

The deterrent to using selenium as a dietary supplement is its inherent toxicity to mammals. There remains a need for nutritional supplements containing amounts of selenium sufficient to confer the prophylactic and therapeutic properties selenium is understood to have, yet not rising to toxic levels.

SAM has been used to treat various disorders. In various forms of liver disease, SAM acts as an anticholestatic agent. [Adachi et al., Japan Arch. Inter. Med., 33:185-192 (1986)]. SAM

has also been administered as an antidepressant for use in the management of psychiatric disorders [Caruso et al., Lancet, 1: 904 (1984)], and as an anti-inflammatory compound in the management of atherosclerosis [Domljan et al., Int. J. Clin. Pharm. Toxicol., 27(7):329-333 (1989)].

Low levels of SAM are believed to play a role in reducing the risk of certain cancers. [Feo et al., Carcinogenesis, 6:1713-20 (1985)]. In addition, the administration of SAM has also been associated with a fall in the amount of early reversible nodules and the prevention of the development of late pre-neoplastic lesions and hepatocellular carcinomas. [Garcea et al., Carcinogenesis, 8:653-58 (1987)].

Unfortunately, the SAM per se is unstable due to its high reactivity. The relatively recent synthesis of stable salts, however, has made SAM available for research and therapeutic use. [See, e.g., U.S. Patent Nos. 4,990,606 and 5,102,791].

Vitamin B₁₂ is generally known to function as a coenzyme in biochemical reactions such as the synthesis of propionic acid and of methionine. Recent evidence suggests that the elevated levels of plasma homocysteine increase the risk of occlusive vascular disease. Adequate amounts of vitamin B₁₂ are considered the most important environmental influence on the accumulation of unnecessary homocysteine. [Joosten et al., Am. J. Clin. Nutr., 58(4): 468-76 (1993)]. In addition, it is also understood that vitamin B₁₂ may play a role in the methylation of selenium. [Chen and Whanger, Tox. and Appl. Pharm., 118:65-72 (1993)]. Specifically, increased levels of vitamin B₁₂ significantly contribute to selenium methylation and might decrease overall

selenium toxicity by preventing its accumulation in tissues.
[Chen and Whanger, cited above].

Cyanide was reported to inhibit tumor cell proliferation in mice with melanoma and in rat hepatoma and human colon cancer cells in culture. [Hu et al., Biochem. Pharm., 37(11):2259-66 (1988)]. In addition, studies have shown that pretreatment with cyanide in experimental animals profoundly inhibited the toxic effects of selenium [Davis et al., Ciba Found. Symp., 140:219-231 (1988)]. On the other hand, cyanide reduces liver glycogen, implying greater emphasis on anaerobic metabolism through inhibition of cytochrome oxidase. This may increase reductive potential, but may also result in increased free radical production. [Davis et al., cited above].

There remains a need in the art for nutritional supplements that can improve mammalian health by virtue of the inclusion of substantially non-toxic amounts of selenium and SAM.

SUMMARY OF THE INVENTION

The present invention relates to the use of nutritional supplement compositions to overcome certain nutritional deficiencies typically associated with the normal Western diet.

It is therefore an object of the invention to provide novel, nutritious, and safe compositions for mammalian and human consumption as dietary supplements that include compendial grades of selenium and SAM, and that optionally include compendial grades of vitamin B₁₂ and cyanide.

It is another object of the invention to provide methods of administering the nutritional supplements of the present

invention to mammals.

As described above, the methylated forms of selenium are understood to confer certain health benefits beyond those of unmethylated forms of selenium. Therefore, it is yet another
5 object to provide a method of increasing the methylation of selenium.

DETAILED DESCRIPTION OF THE INVENTION

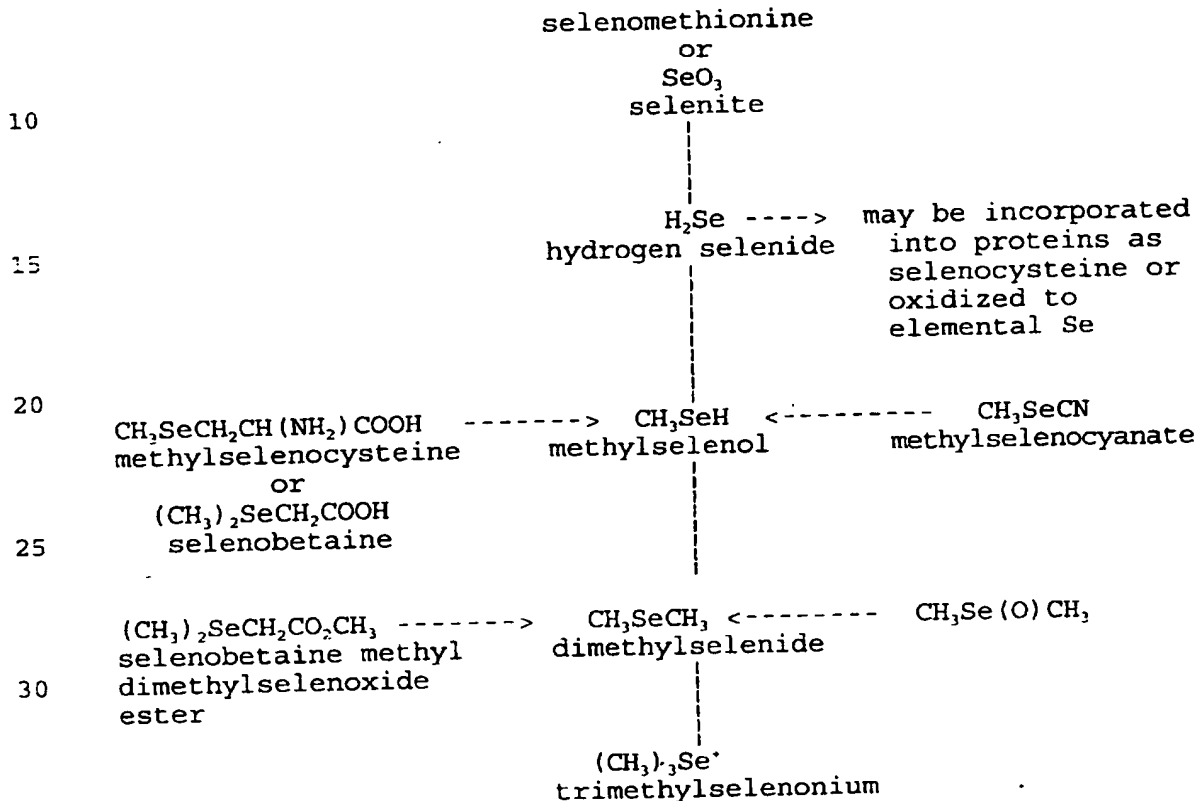
The novel nutritional compositions of the present invention provide compounds that are understood to improve the health of
10 mammals. The present compositions are novel combinations of naturally occurring substances, are substantially non-toxic when administered according to the methods of the present invention, and provide for a more complete nutritional regime upon administration.

15 The present invention focuses on the development and maintenance of vitality and fortitude of mammals as a direct result of the oral intake of the combination of compositions of the present invention. A primary aim of the present invention is to provide compositions that act on the mammalian systems to
20 safely reduce the risks of health problems, including those arising from the presence of oxidants in the mammalian blood and tissues. This goal is accomplished by providing nutritional supplements that include selenium and SAM, the SAM component acting to increase the degree of selenium methylation, thus
25 increasing its bioactivity.

The present invention accomplishes these goals by providing, in amounts sufficient to confer the desired health benefits, a

combination of a compendial grade of selenium and a compendial grade of SAM in a nutritional supplement composition. Optionally, a compendial grade of vitamin B₁₂ and/or cyanide can be added to the nutritional supplements of the present invention.

The metabolic pathway of selenium is understood to be as follows:



35 The metabolic detoxification of selenium requires a
methylation pathway for its two major metabolites, dimethyl
selenide and trimethylselenonium ion. In this metabolic pathway,
an inorganic form of selenium called selenite undergoes reduction
to form hydrogen selenide. This active intermediary metabolite
40 may be used for selenoprotein synthesis, oxidized to elemental
selenium or methylated into monomethyl, dimethyl, and trimethyl

metabolites. The process of methylation is a detoxification mechanism.

With high intakes of selenium, the levels of intermediate metabolites increase, particularly methylated derivatives. Generally, it is understood that the monomethylated form of selenium, methylselenol, possesses the highest biological activity. Trimethylselenonium is inactive, most likely because it is rapidly excreted in urine. Dimethylselenide is usually exhaled.

The importance of this pathway is that good anticarcinogenic activity is recognized when selenium is pushed beyond the hydrogen selenide step in the process -- this results in the creation of methylselenol, which has been proposed as the likely candidate for cancer inhibition. [Lu et al., Biochem. Pharm., 50(2):213-219 (1995)].

The nutritional supplement compositions of the present invention contain a dosage of about 10 μ g to about 5 mg, and preferably about 800 μ g of selenium.

Normal selenium metabolism involves reduction with glutathione and methylation by SAM, the body's primary biologic methyl donor. Since the methylated forms of selenium have increased biological activity, selenium compounds which generate a stream of methylated metabolites are more effective for consumption.

In the body, SAM is synthesized from methionine and ATP by S-adenosylmethionine synthetase (ATP-L-methionine-S-adenosyltransferase). Unfortunately, however, administration of SAM cannot be replaced by the administration of methionine.

During more than ten years of clinical use, as well as short-term, subacute, and long-term studies in animals, no serious toxic response from the use of SAM has been reported. On the other hand, methionine is highly toxic and may cause disorientation, vomiting, and shock with liver damage. This high toxicity may be the result of the rise in production of highly toxic circulating mercaptans resulting from the limited capabilities of mammals to metabolize methionine to SAM.

According to the present invention, the simultaneous administration of selenium and SAM would result in increased utilization of SAM to convert selenium to its methylated derivatives. In addition, because the substitution of selenium for sulfur in SAM renders SAM more bioactive, the simultaneous administration of selenium and SAM increases the methylating activity of SAM. Thus, selenium and SAM are believed to function in concert in the nutritional supplement compositions of the present invention.

It is known that the rate of demethylation of trimethylselenonium is dependent on homocysteine. Since homocysteine is in the metabolic pathway of SAM, the inclusion of SAM in the nutritional supplement compositions of the present invention will have the dual functions of increasing the methylation of selenium as well as demethylating the end metabolite of trimethylselenonium, through the increase in homocysteine, and, thus, preserving higher levels of selenium in a more active anticarcinogen form. This may also result in a reduction of the dose of selenium required in the nutritional supplements of the invention to achieve the same biological

effects as produced by a dosage of selenium without SAM.

The dosage of SAM in the nutritional supplements of the present invention ranges from about 5 mg to about 5000 mg, and preferably about 2000 mg. The dosage of selenium in the
5 nutritional supplements of the present invention ranges from about 10 μ g to about 5 mg, and preferably about 800 μ g.

Vitamin B₁₂ acts as a co-factor in methylation. In a preferred embodiment, a compendial grade of Vitamin B₁₂ may be employed in a multi-vitamin component. Each multi-vitamin
10 component of such an embodiment comprises, by weight, about 0.1 mg to about 10.0 mg of Vitamin B₁₂, preferably about 5 mg.

The fourth component in a preferred embodiment of the invention is cyanide. As stated above, cyanide may function as to inhibit the toxic effects of selenium. However, also as
15 stated above, the presence of cyanide may increase the levels of oxidants in living systems, and the antioxidant effect of selenium is expected to ameliorate this side effect of cyanide. Hence, the nutritional supplement compositions of the present invention may optionally include a compendial grade of naturally
20 occurring and substantially non-toxic cyanide in an amount sufficient to inhibit the toxic effects of selenium. In the nutritional supplement compositions of the present invention, a compendial grade of cyanide may be present in that amount of about 1 mg to about 1000 mg, and preferably about 500 mg.

25 As a preferred embodiment, a dosage of the nutritional supplement compositions of the present invention may consist of a capsule for human oral consumption. In such an embodiment, the preferred weight of the dosage is between about 5 mg to about

5000 mg, and preferably about 2500 mg. The dosage may be administered in a single daily dosage form, e.g., a capsule of preferably 2500 mg. Alternatively, the nutritional supplement compositions of the present invention may be administered more than once daily. Hence, the presently claimed nutritional supplement compositions may be in the form of an oral dosage form of 1250 mg administered twice daily or 833 mg administered three times daily.

These preparations may be made by conventional methods. To prepare the compositions of the invention, the above-described selenium and SAM (and optionally vitamin B₁₂ and/or cyanide) are combined as the active ingredient in intimate admixture with a suitable carrier according to conventional compounding techniques. This carrier may take a wide variety of forms depending upon the form of preparation desired for administration, e.g., oral, sublingual, nasal, or parenteral.

In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed. For oral liquid preparations (e.g., suspensions, elixirs, and solutions), media containing for example, water, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used. Carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be used to prepare oral solids (e.g., powders, capsules, pills, caplets, tablets, and lozenges). Capsules are a preferred oral dosage form. Controlled release forms may also be used. Because of their ease in administration, lozenges, tablets, pills, caplets, and capsules represent the most advantageous oral dosage

unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar coated or enteric coated by standard techniques.

For parenteral products, the carrier will usually comprise
5 sterile water, although other ingredients may be included, e.g.,
to aid solubility or for preservation purposes. Injectable
suspensions may also be prepared, in which case appropriate
liquid carriers, suspending agents, and the like may be employed.

Numerous modifications and variations of the present
10 invention are included in the above-identified specification and
are expected to be obvious to one of skill in the art. It is
also intended that the present invention cover modifications and
variations of the compositions and method for using them to
accomplish their claimed uses within the scope of the appended
15 claims and their equivalents.

WE CLAIM:

1. A nutritional supplement comprising selenium and s-adenosylmethionine.

2. The nutritional supplement of claim 1, wherein the
5 selenium is between approximately 10 μ g to approximately 5 mg, and the s-adenosylmethionine is between approximately 5 mg to approximately 5000 mg.

3. The nutritional supplement of claim 2, wherein the selenium is approximately 800 μ g.

10 4. The nutritional supplement of claim 2, wherein s-adenosylmethionine is approximately 2000 mg.

5. The nutritional supplement of claim 1, further comprising vitamin B₁₂.

6. The nutritional supplement of claim 5, wherein the
15 selenium is between approximately 10 μ g to approximately 5 mg, the s-adenosylmethionine is between approximately 5 mg to approximately 5000 mg, and the vitamin B₁₂ is between approximately 0.1 mg to approximately 10 mg.

7. The nutritional supplement of claim 6, wherein the
20 selenium is approximately 800 μ g.

8. The nutritional supplement of claim 6, wherein the s-adenosylmethionine is approximately 2000 mg.

9. The nutritional supplement of claim 6, wherein the vitamin B₁₂ is approximately 5 mg.

25 10. The nutritional supplement of claim 1, further comprising cyanide.

11. The nutritional supplement of claim 10, wherein the

selenium is between approximately 10 µg to approximately 5 mg, the s-adenosylmethionine is between approximately 5 mg to approximately 5000 mg, the cyanide is between approximately 1 mg to approximately 1000 mg, and the vitamin B₁₂ is between approximately 0.1 mg to approximately 10 mg.

12. The nutritional supplement of claim 11, wherein the selenium is approximately 800 µg.

13. The nutritional supplement of claim 11, wherein the s-adenosylmethionine is approximately 2000 mg.

14. The nutritional supplement of claim 11, wherein the cyanide is approximately 500 mg.

15. The nutritional supplement of claim 11, wherein the vitamin B₁₂ is approximately 5 mg.

16. A method of preparing nutritional supplement comprising the step of admixing selenium and s-adenosylmethionine components with a suitable carrier.

17. A method of preparing a nutritional supplement comprising the step of admixing selenium, s-adenosylmethionine, and vitamin B₁₂ with a suitable carrier.

18. A method of preparing a nutritional supplement comprising the step of admixing selenium, s-adenosylmethionine, cyanide, and vitamin B₁₂ with a suitable carrier.

19. A method of administering the nutritional supplement of any of claims 1, 4 or 7.

20. A method of increasing the methylation of selenium comprising the step of contacting selenium with s-adenosylmethionine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16366

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H19/167; A61K31/095, 33/04; A01N 59/02

US CL : 424/702; 514/706; 536/27.31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/702; 514/706; 536/27.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS,CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	McGARRITY T.J. Effect of Selenium on Growth, S-Adenosylmethionine and Polyamine Biosynthesis in Human Colon Cancer Cells. Anticancer Research, 1993, Vol. 13, pages 811-816	1-4,16,20
Y	DAUSCH J.D. Increased Levels of S-Adenosylmethionine in the Livers of Rats Fed Various Forms of Selenium. Nutr. Cancer, 1993, VOL.20, pages 31-39	1-4,16,20
Y	CHEN C.L. Effect of Vitamin B12 Status on Selenium Methylation and Toxicity in Rats. Toxicol. Appl. Pharmacol. 1993, VOL.118, pages 65-72.	5-9,17

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 NOVEMBER 1997

Date of mailing of the international search report

4 JAN 1998

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/16366

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEN C. Effect of Vitamin B12 on Performance and Tissue Selenium Content in Rats Fed Sub-toxic Levels of Selenite. Toxicology, 1993, VOL.85, pages 101-115.	5-9,17
Y	DAVIS R.H. Nutritional and Biochemical Factors Influencing the Biological Effects of Cyanide. Ciba Found. Symp., 1988, VOL. 140, pages 219-231	10-15,18
A	US 5,084,482 A (HIRSCH et al.)28 January 1992, col. 6, lines 10-20, claim 7.	1-9,16, 17,20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16366

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16366

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-19, drawn to compositions, one method of making and one method of use.
Group II, claim 20, drawn to the second method of use.

The method of use included in Group I and the method of use of Group II do not share a special technical feature because they are drawn to method of in vivo administration of a nutrition supplement and to the method of methylation of selenium, respectively.